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# Increased severity of fragile X spectrum disorders in the agricultural community of Ricaurte, Colombia

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ABSTRACT

Premutation carriers of the *FMR1* gene (CGG repeats between 55 and 200) usually have normal intellectual abilities but approximately 20% are diagnosed with developmental problems or autism spectrum disorder. Additionally, close to 50% have psychiatric problems such as anxiety, ADHD and/or depression. The spectrum of fragile X disorders also includes Fragile-X-associated primary ovarian insufficiency (FXPOI) in female carriers and Fragile-X-associated tremor/ataxia syndrome (FXTAS) in older male and female carriers.

We evaluated 25 premutation carriers in the rural community of Ricaurte Colombia and documented all behavioral problems, social deficits and clinical signs of FXPOI and FXTAS as well as reviewed the medical and obstetric history.

We found an increased frequency and severity of symptoms of fragile X spectrum disorders, which might be related to the vulnerability of *FMR1* premutation carriers to higher exposure to neurotoxic pesticides in this rural community.

## 1. Introduction

Fragile X syndrome (FXS) is a disease of genetic origin that has a prevalence of 1: 5000 men and 1: 4000–8000 women, while the prevalence of carrying the premutation is estimated at 1: 400–850 males and 1 in 150–300 females (Seltzer et al., 2012; Hunter et al., 2014; Tassone et al., 2012). Inherited through the X chromosome, FXS is caused by the unstable expansion of the CGG triplet in the gene Fragile X Mental Retardation 1 (*FMR1*). Among individuals with the full mutation of *FMR1* (> 200 CGG repeats), 60% of males are diagnosed with Autism Spectrum Disorders (ASD), and almost all males and 30% of females have intellectual disability (ID) (Saldarriaga et al., 2014). Female carriers of the premutation (CGG repeats between 55 and 200) can pass the mutation to 50% of their offspring, although in many cases, offspring experience expansion of the CGG repeats to the full mutation resulting in FXS. Children with the premutation usually have normal intellectual abilities but approximately 20% are diagnosed with developmental problems or autism spectrum disorder (ASD), which are part of the spectrum of fragile X disorders (FXSD) (Farzin et al., 2006; Lozano et al., 2014). Psychiatric problems such as anxiety, ADHD and/or depression are present in approximately 50% of children and adults with the premutation (Farzin et al., 2006; Bourgeois et al., 2011; Cordeiro et al., 2015; Klusek et al., 2017).

Additional disorders included in FXSD include Fragile-X-associated primary ovarian insufficiency (FXPOI), characterized by infertility and menopause prior to age 40, which occurs in approximately 20% of female carriers (Cronister et al., 1991; Sullivan et al., 2011); in contrast, premature ovarian insufficiency (POI) is observed in 1% of the general population (Sherman, 2000). In addition, 12.6% of female *FMR1* premutation carriers without FXPOI have difficulty getting pregnant

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(Wheeler et al., 2014). Fragile-X-associated tremor ataxia syndrome (FXTAS) is a neurodegenerative disorder that occurs in some older carriers and involves an intention tremor and cerebellar ataxia. FXTAS was originally described in male carriers of the premutation who were older than 50 years (Hagerman et al., 2001). Later it was found to affect approximately 40% of males but also female premutation carriers with a penetrance of 16% (Hall and O'keefe, 2012; Rodriguez-Revenga et al., 2009; Jacquemont et al., 2004). Seizures occur in less than 1% of female premutation carriers and approximately 8–14% of male premutation carriers (Bailey et al., 2008; Chonchaiya et al., 2012).

Experimental studies have revealed that premutation neurons from the knock-in mouse die more readily in cell culture compared to neurons without the premutation (Chen et al., 2009), an observation that has given rise to the hypothesis that *FMR1* premutation increases the vulnerability of neurons to environmental toxins (Hagerman and Hagerman, 2013). Neurological deterioration in patients with *FMR1* premutation has been associated with exposure to many environmental toxins, including chemotherapeutic agents (O'Dwyer et al., 2005), anesthetics (Ligsay et al., 2018), substance abuse of opioids and or alcohol (Muzar et al., 2014, 2015; El-Deeb et al., 2018), and perhaps phenobarbital (Saldarriaga et al., 2016).

Ricaurte, a small town in Valle del Cauca, province of Colombia, has severe economic limitations. The base of the economy is agriculture and cattle raising, which are practiced without the aid of industrial equipment and often under precarious working conditions. Ricaurte, population of less than 1500 people, has a cluster of FXS, with 1:19 men and 1:46 women carrying the full mutation, and 1:85 men and 1:25 women carrying the premutation (Saldarriaga et al., 2018). We evaluated all premutation carriers to assess the frequency and severity of FXSD including FXTAS, FXPOI, seizures and behavioral problems.

## 2. Materials and methods

This study was conducted from January 2016 to May 2016. The protocol was reviewed and approved by the Institutional Review Board (IRB) of the School of Health of Universidad del Valle (Cali, Colombia). We evaluated all premutation carriers, 20 women and 5 men, age range between 11 months and 83 years, all of whom are of Hispanic ethnicity. To be included in the study subjects had to be a native of Ricaurte, Colombia and diagnosed as being a premutation carrier using polymerase chain reaction (PCR) and Southern blot to quantify the number of CGG repeats in the *FMR1* gene (Saldarriaga et al., 2018). All patients signed an informed consent authorizing an in-depth review of their clinical history including medical problems and reproductive issues as well as a physical evaluation that included the use of standardized rating scales and interview-based measures, specifically, the Scale for the Assessment and Rating of Ataxia (SARA) (Schmitz-Hübsch et al., 2006), and the Fahn-Tolosa-Marín Clinical Rating Scale for Tremor (FTM) (Fahn et al., 1993) to assess neurological findings during the exam of those carriers older than 50 years of age (n = 14). SARA is a valid clinical rating scale administered by the physician that has proven to be significantly correlated with results obtained using the International Cooperative Ataxia Rating Scale (ICARS) (Yabe et al., 2008) and the Modified Barthel Index (MBI) (Weyer et al., 2007; Kim et al., 2011). FTM is the most commonly used scale to evaluate essential tremor; it assesses postural, kinetic and rest tremor (Ondo et al., 2018). General mental status was measured by the raw total score on the Mini Mental State Exam (MMSE) (Folstein et al., 1975) and the Short Test of Mental Status (STMS) (Kokmen et al., 1987, 1991) was used to explore mild cognitive impairment; however, the calculation subpart was left out since most of the older participants did not have the educational level required to perform the tasks. Executive abilities were evaluated with the Behavioral Dyscontrol Scale (BDS) (Grigsby and Kaye, 1996; Grigsby et al., 1992). BDS was previously used to report dysexecutive syndrome in carrier males with FXTAS (Brega et al., 2008).

All carriers were also evaluated for behavioral problems including anxiety and depression, as well as social deficits, including social anxiety, as determined using the Generalized Anxiety Disorder 7 (GAD-7) scale (Spitzer et al., 2006), the Mayor Depression Inventory (MDI) (Bech, 2012) and, the Brief Fear of Negative Evaluation (BFNE) scale (Leary, 1983), respectively. GAD-7 measures severity of anxiety, it has an overall sensibility of 89% and specificity of 82% (Spitzer et al., 2006; Kroenke et al., 2007); MDI is a self-report mood questionnaire developed by the WHO, and BFNE is a self-report questionnaire that measures the cognitive component of social anxiety (Carleton et al., 2006; Weeks et al., 2005). Despite being self-report questionnaires, all surveys were administered by the physician as interview-based measures in order to overcome the educational barrier encountered with most of the participants. Further explanation was offered when needed. In the case of one participant who had mutism and ID, the assessments were answered by her sister. One participant, who was an infant, was excluded from the assessment. We did not measure IQ since most premutation carriers have normal IQ (Hagerman and Hagerman, 2013; Myers et al., 2001; Grigsby et al., 2014).

## 3. Results

### 3.1. FXTAS

All of the female premutation carriers in the study population were evaluated (n = 20). Twelve of them (60%) were 50 years of age or older. These twelve patients underwent a further clinical evaluation, including a neurological examination, using SARA and FTM to assess the presence and severity of ataxia and tremor. Two subjects (16.6%) had ataxia and two (16.6%) had tremor; none had both. Out of the 5 male carriers, two were over 50 years old; one had a distal tremor without ataxia and the other had no tremor or ataxia, but presented with executive dysfunction, which is a FXTAS-associated cognitive deficit (Brega et al., 2008; Hagerman and Hagerman, 2016) (see Table 1).

### 3.2. FXPOI

Sixteen (80%) out of the twenty female premutation carriers were older than 40 years of age, and were asked to answer a questionnaire regarding gynecological and reproductive issues including infertility and early menopause. Three (18.7%) had absence of their menstrual cycle for more than a year before reaching 40 years, which meets the absolute criterion of FXPOI. Of the three women who met this criterion, one has two sons affected with FXS, one has three children, one of whom is a carrier of the premutation allele, and the third has no children. In addition, from the remaining 13 premutation carriers older than 40 years of age, one woman underwent hysterectomy at 35 years old due to persistent vaginal bleeding associated with perimenopause symptoms (headache, hot flashes, mood swings) and three others had a gravida 0, para 0 (G0P0) obstetric history despite having a sexual partner and seeking reproduction by natural methods. Therefore a total of four women (25%) had findings suggestive of ovarian insufficiency (abnormal bleeding and reproductive issues) in addition to the 3 women with FXPOI (see Table 1).

### 3.3. Seizures

Five (25%) out of twenty female premutation carriers presented with seizures, most of them since childhood, with poor control and noticeable cognitive decline. Two of them also presented symptoms of FXTAS before age 50; one with cerebellar atrophy and one with cerebral atrophy reported on clinical MRI (Saldarriaga et al., 2016). Four of the five subjects with seizures had concomitant symptoms of ovary insufficiency (see Table 1).

**Table 1**
Summary of Results.

| Age | Gen | Allele | Exp ** | LOE | Obstetric History | Children with FM | Children with PM | Menopause (Age) | FXPOI | FXTAS | Seizures | Cognit | Anxiety | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 71 | F | 30,57 | Y | 40 | G0P0 | 0 | 0 | 39 | * | | | | | |
| 38 | F | 30,55 | N | | G3P3L3 | 0 | 1 (M) | NO | | | | | | |
| 44 | F | 30, 142 | Y | 15 | G2P2 | 2 (M) | 0 | 38 | * | | | | * | Mild anxiety |
| 54 | F | 30,55 | N | | G4P5L4 | 0 | 1 (F) | 51 | | | | | | |
| 53 | F | 30,69 | Y | 10 | G0P0 | 0 | 0 | 51 | | | | * | | Mutism |
| 60 | F | 30,110 | Y | 30 | G0P0 | 0 | 0 | 55 | | | * | | | Cerebral atrophy |
| 54 | F | 45,135 | Y | 20 | G1C1 | 1 (M) | | 50 | | * | * | | | Cerebellar atrophy, ataxia started at 42 yo |
| 80 | F | 29,86 | Y | 35 | G9P9 | 3 (M) 1 (F) | 1 (F) | 51 | | * | | | | Tremor started at 75 yo |
| 68 | F | 29,105 | Y | 15 | G4P4 | 1 (M) | 1 (F) | 57 | | | | | | |
| 53 | F | 30,82 | Y | 25 | G5P3C2L2 | 0 | 0 | 35 | | * | * | | * | Cerebral atrophy, hysterectomy 35 yo, moderate anxiety, tremor started at 46 yo |
| 26 | F | 39,77 | N | | G2C1L0 | 0 | 0 | NO | | | | | | |
| 71 | F | 30,67 | Y | 40 | G0P0 | 0 | 0 | 50 | | | * | * | * | Intellectual disability, severe anxiety |
| 49 | F | 30,79 | N | | G2P2 | 1 (M) | 1 (F) | 47 | | | | | | |
| 47 | F | 30,82 | N | | G1P1 | 0 | 0 | NO | | | | | | |
| 57 | F | 30,80 | N | | G2P2 | 2 (M) | 0 | 48 | | | | | | |
| 47 | F | 30,71 | N | | G3P3L2 | 0 | 0 | 45 | | | | | * | Moderate anxiety |
| 39 | F | 30,66 | Y | 21 | G3P2C1 | 0 | 1 (M) | 36 | * | | * | | | |
| 65 | F | 29,82 | N | | G1P1 | 0 | 1 (F) | 50 | | | | | | |
| 62 | F | 30,121 | Y | 35 | G4P2C2L3 | 2 (F) | 0 | 51 | | | | | | |
| 38 | F | 29,102 | N | | G1P1 | 0 | 0 | NO | | | | | | |
| 11m | M | 55 | N | | 0 | 0 | 0 | NA | | | | | | |
| 16 | M | 55 | N | | 0 | 0 | 0 | NA | | | | | * | Mild anxiety |
| 83 | M | 69 | Y | 40 | 3 (F) 4 (M) | 0 | 3 (F) | NA | | * | | | | Tremor started at 75yo |
| 70 | M | 61 | Y | 40 | 4 (F) 3 (M) | 0 | 3 (F) | NA | | | | * | * | Disinhibition and emotion regulation problems, moderate anxiety |
| 14 | M | 123 | N | | 0 | 0 | 0 | NA | | | | | | |

*All patients are Hispanic. Gen = Gender; Exp = Exposed to pesticides; Y = yes; N = no; LOE = Length of pesticide exposure (in years); Cognit = Cognitive; PM = premutation; FM = full mutation; NA = not applicable; M = male and F = female; G = gravida (number of pregnancies), P = para (number of pregnancies reaching viable gestational age) C = cesarean delivery and L = living children.

** Those exposed have worked in the field, some of them since childhood. The length of exposure to each specific pesticide is unknown since their use depends on the type of crop. Products commonly grown include cotton, milo, tomato, papaya, pineapple and sugar cane. Younger generations have had better educational resources and have transitioned to more diverse job opportunities in nearby cities. However; agriculture remains to be the main economic activity of the region. No one is exempt from exposure one way or another.

### *3.4. Cognition and behavioral problems*

Two female and one male premutation carrier presented with cognitive deficits. Of these three, one presented with lifelong mutism, another one with intellectual disability and the third with executive dysfunction (deficit in behavioral self-regulation); one of the females and the male also presented with moderate to severe anxiety. Of the 25 subjects in the study, 6 individuals (24%) were experiencing symptoms of general and social anxiety at the time of their medical evaluation, which was untreated because these symptoms are not identified by the patients as a medical problem that they brought to the attention of a physician.

## 4. Discussion

In Ricaurte, the frequency and severity of symptoms of FXTAS, FXPOI and seizures in carriers of the *FMR1* premutation are higher than those reported in the literature (Rodriguez-Revenga et al., 2009; Jacquemont et al., 2004; Hagerman and Hagerman, 2013).

We found that in male and female carriers, 50% and 33.3% respectively, of those older than 50 years of age presented symptoms and clinical signs suggestive of FXTAS. Patients with tremor and ataxia had an early onset of symptoms and severity, none of them reported a history of exposure to chronic use of opioids and/or alcohol or general anesthesia; however, all of these carriers worked in agriculture, either in the fields or in their home gardens since childhood, as ascertained from the medical history. We also found that 18.7% of the women who were carriers had FXPOI and 25% of the women without FXPOI had findings suggestive of ovarian insufficiency (see Table 1).

Overexpression of FMR1 mRNA leading to RNA toxicity is thought to be the cause for most clinical problems associated with the FMR1 premutation (Hagerman and Hagerman, 2013, 2016). Neurotoxic effects of elevated cellular *FMR1* mRNA levels leads to sequestration of important proteins for neuronal function, calcium dysregulation, mitochondrial dysfunction, impaired DNA damage repair and formation of intranuclear inclusions in the brain of premutation carriers with FXTAS Hagerman and Hagerman, 2016; Iwahashi et al., 2006). Mitochondrial dysfunction and increased oxidative stress are also observed in brain and tissue samples of premutation carriers both with and without FXTAS (Napoli et al., 2016). These deficits predispose premutation neurons to early death compared to neurons without the premutation (Chen et al., 2009). Furthermore, the mitochondrial deficits and calcium dysregulation in premutation neurons leads to excessive spike discharges and greater vulnerability to toxic environmental contaminants that converge on these same pathways of neurotoxicity, thereby exacerbating neurological symptoms and contributing to increased penetrance of seizures, and symptoms of FXTAS and FXPOI, the latter problem related to toxicity directly to the ovary (Saldarriaga et al., 2016; Ross-Inta et al., 2010; Cao et al., 2012).

The relationship between male carriers and seizures has been described in children. Bailey and colleagues (Bailey et al., 2008) conducted a National Parental Survey to assess co-occurring conditions in

patients with full mutation and premutation and found that 14% of males (8 out of 57 premutation carriers) reported seizures while 1.2% of male controls without the premutation reported seizures. Also, Chonchaiya and colleagues (Chonchaiya et al., 2012) found an increased prevalence of seizures among young premutation boys with comorbid ASD: 1 out of 26 subjects without ASD had seizures (3.85%), whereas 6 out of 24 subjects with ASD had seizures (25%). In female carriers of the premutation, seizures have been described as a rare co-occurring condition: in the National Parental Survey, seizures were observed in 0.5% (1 out of 199) of female premutation carriers and 1.7% of female controls (Bailey et al., 2008).

In Ricaurte, among the women with premutation, 25% (5/20) had seizures, two of them were sisters and the other three were relatives in the fourth degree of consanguinity; all five had a common ancestor. A possible explanation for the origin of the seizures in these five women could be a second genetic hit; however, the two sisters previously had a genome-wide assessment of 2,391,739 SNPs to assess copy number variants (CNVs), and no disease-associated CNVs were found (Saldarriaga et al., 2016). Two of the women also fulfilled the clinical and radiological criteria of FXTAS and an additional one had ASD (see Table 1).

Ricaurte is a small rural town where the base of the economy is agriculture, and the inhabitants are exposed to pesticides through their work in the fields, the common practice of storing pesticides inside their homes and/or contact with contaminated water or food. The most commonly used pesticides in the fields include neonicotinoids, avermectins, organophosphorus compounds and pyrethroids (Oral Communication: Pesticides Used for Agriculture in Ricaurte, 2015). An association of these neurotoxic environmental contaminants with the exacerbation of neurological deterioration in *FMR1* premutation carriers has yet to be described; however, this possibility is supported by experimental evidence demonstrating that these pesticides converge on pathways of neurotoxicity inherent in premutation neurons. Specifically, there is evidence that these classes of pesticides can interfere with neuronal morphogenesis (Howard et al., 2005; Yang et al., 2008, 2011), alter axonal and mitochondrial transport (Middlemore-Risher et al., 2011; Salama et al., 2014; Hernandez et al., 2015), dysregulate calcium signaling in neurons (Cao et al., 2014; Morisseau et al., 2009), induce neuronal apoptosis (Moyano et al., 2017; Raszewski et al., 2014; Caughlan et al., 2004) or inhibit GABA activity (Shelton et al., 2012). Thus, expression of the *FMR1* premutation likely confers increased vulnerability to pesticide neurotoxicity, and interactions between this genetic vulnerability and these pesticides would be predicted to amplify the neurotoxic effects of either factor alone. If true, then exposure to neurotoxic pesticides could explain the increased frequency and severity of clinical symptoms of FXTAS, FXPOI and seizures in carriers of the *FMR1* premutation in this rural population.

## 5. Conclusion

The identification of specific environmental factors that interact with the *FMR1* premutation is warranted since it would enable the development of interventions that would lower the incidence and severity of clinical symptoms. This information may also provide useful insight in identifying genetic subgroups in the population that are more vulnerable to pollution. This should hopefully lead to better laws to protect the general population from this type of pollution.

## Geolocation information

Ricaurte, Valle del Cauca, Colombia, South America

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